

CLAIMS

What is claimed is:

1. A method of pooled screening of cells from different genetic backgrounds, comprising:
 - labeling two or more populations of cells of different genetic backgrounds with two or more unique nucleic acid barcode sequences, each unique nucleic acid barcode sequence corresponding to a different population of cells;
 - combining the two or more populations of cells to obtain a single mixed population of cells in a well on a culture plate;
 - performing *in situ* single-cell sequencing on the cells;
 - obtaining a first plurality of images, wherein the first plurality of images comprises one or more barcoding images of the well on the culture plate;
 - obtaining a second plurality of images, wherein the second plurality of images comprises one or more marker-based or marker-free readout images of the well on the culture plate;
 - generating a first reference coordinate space of the first plurality of images;
 - extracting a first patch of the first plurality of images;
 - generating a second reference coordinate space of the second plurality of images;
 - extracting a second patch of the second plurality of images;
 - computing an affine transformation function between the first patch and the second patch to obtain a plurality of transformation parameters; and
 - generating a coordinate transformation function between the first reference coordinate space and the second reference coordinate space based on the plurality of transformation parameters;
 - identifying cell-specific image data for one or more cells in the single mixed population of cells by correlating at least one image of the first plurality of images with at least one image of the second plurality of images based on the coordinate transformation function; and
 - identifying one or more phenotypes of the one or more cells based on the cell-specific image data.

2. The method of claim 1, wherein the first plurality of images and the second plurality of images provide different coverage of the well, are taken at different times, and/or have different resolutions.
3. The method of claim 1 or 2, wherein the first plurality of images is captured by a first imager.
4. The method of claim 3, further comprising:
 - detecting one or more physical characteristics of the well in the first plurality of images, wherein the one or more physical characteristics of the well comprises: a shape of the well, an edge of the well, a location of the well; and
 - generating the first reference coordinate space based on the detected one or more physical characteristics.
5. The method of claim 3 or 4, wherein two or more images in the first plurality of images are offset from each other by an overlap ratio, and wherein the first reference coordinate space is generated based on the overlap ratio.
6. The method of any one of claims 2-5, further comprising: selecting one or more marker images from the first plurality of images based on one or more landmarks captured in the one or more images, wherein the first patch is obtained from the one or more marker images from the first plurality of images, and wherein the one or more landmarks comprise: one or more cells, one or more well boundaries, one or more beads, one or more nuclei, or any combination thereof.
7. The method of any one of claims 1-6, wherein the second plurality of images captured by a second imager.
8. The method of claim 7, further comprising:
 - detecting one or more physical characteristics of the well in the second plurality of images, wherein the one or more physical characteristics of the well comprises: a shape of the well, an edge of the well, a location of the well; and

generating the second reference coordinate space based on the detected one or more physical characteristics.

9. The method of claim 7 or 8, wherein two or more images in the second plurality of images are offset from each other by an overlap ratio, and wherein the second reference coordinate space is generated based on the overlap ratio.

10. The method of any one of claims 7-9, further comprising: selecting one or more marker images from the second plurality of images based on one or more landmarks captured in the one or more images, wherein the second patch is obtained from the one or more marker images of the second plurality of images, and wherein the one or more landmarks comprise: one or more cells, one or more well boundaries, one or more beads, one or more nuclei, or any combination thereof.

11. The method of any one of claims 1-10, wherein the first patch covers a center of the first image and/or wherein the second patch covers a center of the second image.

12. The method of any one of claims 1-11, wherein at least a portion of the first patch and at least a patch of the second patch correspond to the same subject.

13. The method of any one of claims 1-12, wherein the transformation parameters comprise one or more of: a translation parameter, a scaling parameter, and a rotation parameter.

14. The method of any one of claims 1-13, further comprising utilizing a classifier configured to receive an image and output a classification result.

15. The method of claim 14, wherein the classification result is a classification based on the genetic background of each single cell captured in the image.

16. The method of claim 14 or 15, further comprising generating an embedding of the image.

17. The method of claim 16, wherein the embedding is reduced in dimension using a UMAP (Uniform Manifold Approximation and Projection for Dimension Reduction) algorithm to obtain one or more UMAP plots for visualization of the embedding.
18. The method of claim 16 or 17, further comprising evaluating a treatment based on at least a portion of the embedding.
19. The method of claim 17 or 18, further comprising evaluating a treatment based on at least a portion of the one or more UMAP plots.